

# The first two hotspots of subterranean biodiversity in South America

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## Abstract

The term hotspots of subterranean biodiversity has been used to define subterranean habitats with an arbitrary cutoff of twenty or more obligate stygobitic and troglobitic species. Until present, no hotspots of subterranean biodiversity had been identified in South America. Thus, the objective of this work is to present the first two hotspots of subterranean biodiversity in that continent. The two hotspots of subterranean biodiversity are the Toca do Gonçalves cave (22 spp.) and Areias cave systems (28 spp.). The cave species, some of them considered relict species, belong to the Platyhelminthes (1 sp.), Nemertea (1 sp.), Gastropoda (2 spp.), Amphipoda (2), Isopoda (7), Decapoda (1), Collembola (5), Coleoptera (5), Ensifera (1), Sternorrhyncha (1), Zygentoma (1), Diplopoda (6), Chilopoda (5), Araneae (2), Opiliones (1), Palpi-gradi (2), Pseudoscorpiones (4), and Osteichthyes (2). Although both caves, together, have 50 troglobitic species, only 38% of these species are formally described. Both caves have perennial water bodies, but terrestrial obligate cave invertebrates are dominant in number of species in both systems (around 77%). While the Areias system is partially contained in a conservation unit, Toca do Gonçalves cave is currently unprotected, although it certainly deserves protection.

## Keywords

Cave conservation, cave diversity, protection strategies

## Introduction

The syntagm “biodiversity hotspot”, one of the most important global tools for nature conservation, has been used in different ways, but with the same goal: identifying areas around the world that have high biological diversity (Myers 1988, Reid 1998, Hughes et al. 2002, Brooks et al. 2006). Biodiversity hotspots were defined by Myers et al. (2000) as the biologically richest places with high numbers of species found nowhere else and that have already lost 70% of their original vegetation. Culver and Sket (2000) used the term hotspots of subterranean biodiversity to define subterranean habitats with an arbitrary cutoff of twenty or more obligate stygobitic and troglobitic species, but they did not consider any threats to biodiversity loss. Culver and Pipan (2009) following the concept of the hotspots of subterranean biodiversity, adding another 16 localities to those 20 previously defined by Culver and Sket (2000). Although caves may have a lower diversity when compared to surface habitats, their subterranean fauna have attracted attention because of their biological singularity, high endemism, and evolutionary significance (Culver and Pipan 2009, Chertoprud et al. 2016, Glanville et al. 2016).

Until present, no hotspots of high richness of strictly subterranean species were identified in the Neotropical region (Trajano and Bichuette 2010, Culver and Pipan 2013, Souza-Silva et al. 2015). Since most of the karstic areas of the world are situated in temperate regions, a higher cave biodiversity can be expected in mid-latitudes because caves have served as refugia for many ancestors of obligate cave faunas during strong climate changes in the past (Bar 1968, Romero 2009). However, the high numbers of new species recently discovered in Brazilian caves (Prevorčnik et al. 2012, Ázara and Ferreira 2013 and 2014, Fišer et al. 2013, Iniesta et al. 2012, Iniesta and Ferreira 2013, 2013a, 2013b and 2015, Bastos-Pereira and Ferreira 2015, Vasconcelos and Ferreira 2016) and their high degree of troglomorphism indicate that the events of climatic changes in the Neotropics, even if not so severe as in temperate regions, could have led to the isolation of subterranean lineages. Alternatively, other mechanisms of isolation (e.g. parapatric speciation, oceanic introgressions and regressions) might have led to the evolution of many lineages of subterranean fauna in Brazil (Ferreira et al. 2010, Fišer et al. 2013, Leal-Zanchet et al. 2014). Moreover, most of the caves, particularly in tropical countries, have not been thoroughly explored (Deharveng and Bedos 2000, Lienhard and Ferreira 2015, Souza-Silva and Ferreira 2015).

Despite the fact that South America has the lowest proportions of karstic landscapes of the world, recent studies have shown a high potential for the occurrence of a large number of obligate cave species (Trajano and Bichuette 2010, Deharveng 2005, Deharveng and Bedos 2012, Souza-Silva et al. 2015). The great majority of these recent studies has been developed in Brazil, where the highest proportions of karstic landscapes of South America occur in different rock types (Souza-Silva et al. 2011, CEBS-UFLA 2015, Cordeiro et al. 2014, Gallão and Bichuette 2015).

One of the main tools to predict and avoid future rapid deterioration of subterranean ecosystems may be such studies on endemic cave fauna (Gibert and Deharveng

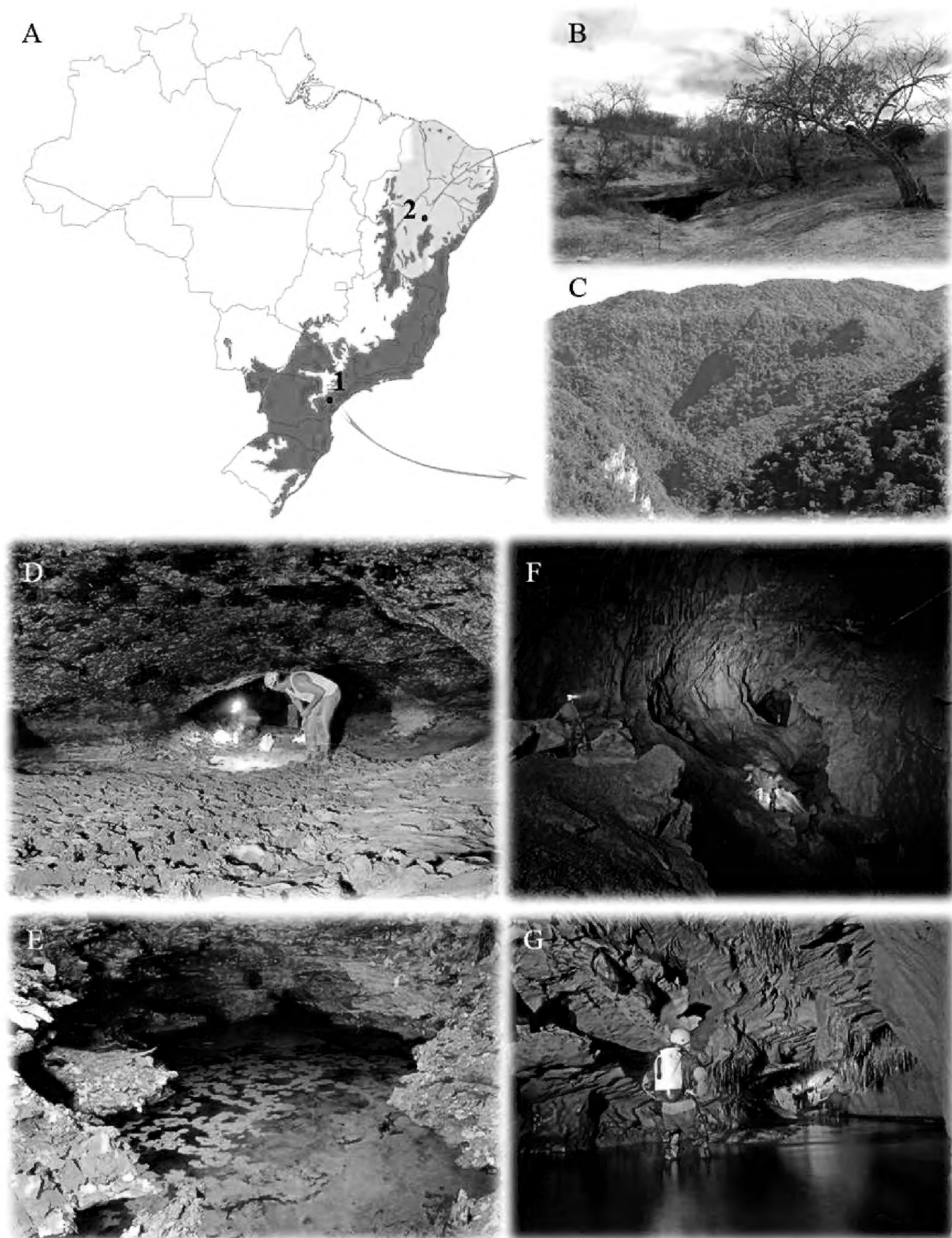
2002). Thus, researches in critical habitats represent an urgent task for scientists, consultants, and policy-makers regarding conservation. Global assessments of the distribution and changes in biodiversity in spatial and temporal contexts have been playing a crucial role in efficiently allocating limited resources to high-priority areas and species (Amano and Sutherland 2013). In this context, the objective of the present work is to present the first two hotspots of subterranean biodiversity in South America, describing their taxonomic biodiversity, organic resources availability and threats, as well as discussing the potential occurrence of other hotspots or important subterranean ecological sites in order to contribute to their conservation.

## **Material and methods**

### **Study area**

The study was conducted in the Areias Cave System (AS) formed by three caves connected by a stream (UTM 22J, 732863/7279192, 100 km from the sea and at 500 m, asl) and Toca do Gonçalves cave (TG) including a water table level (UTM 24L, 292651/8837465, 460 km from the sea and at 527 m, asl) (Figure 1). The Areias system is a 14 km long cave including all the subterranean stretches of the drainage and it is divided into the upstream Areias de Cima cave (5.5 km long and 43 m elevation difference), Areias de Baixo cave (1.5 km long and 20 m elevation difference), and downstream Resurgência das Areias cave (1.2 km long and 73 elevation differences). The Areias system is a convergent drainage of many autogenic and allogenic drainages, also connected with many closed dolines that receive meteoric water. This cave system has terrestrial humid areas and ponds that receive water from the epikarstic/vadose compartments (Genthner et al. 2003). Between Areias de Cima and Areias de Baixo caves there is a sinkhole with a diameter of 140 m and dense arboreal vegetation. Between Areias de Baixo and Resurgência das Areias caves there is a long passage still not explored and limited by river siphons (approximately 5.6 km - J.A. Ferrari pers. comm.). The Areias cave system is located in the municipality of Iporanga, São Paulo state, Southeastern of Brazil (Figure 1). It is protected inside the conservation unit Parque Estadual Turístico do Alto Ribeira (PETAR) in a humid subtropical zone covered by Atlantic Rain Forest that does not have a dry period (Álvares et al. 2014).

The Toca do Gonçalves cave is smaller and located in a semiarid zone (Caatinga formation) in the municipality of Campo Formoso, Bahia state, Northeast of Brazil (Figure 1), and comprises a maze-cave which totals nearly 500 mapped meters. This cave has a single horizontal lenticular-shaped entrance; the maximum dimensions are approximately 2 m high and 8 m width. After the entrance, a rectilinear conduit descends slightly, reaching some of the lateral conduits. The cave has two main branches: the right branch reaches the lower water table while the left branch has predominantly dry and high galleries without macro-access to the water table level.



**Figure 1.** Areias cave system (**F, G**) in the Atlantic Rain Forest (**C**) and Toca do Gonçalves Cave (**A, D, E**) in Caatinga (**B**). Photos **F** and **G** by Daniel Menin.

### Laboratory and field procedures to Toca do Gonçalves and Areias cave system

The database of the obligate cave fauna was obtained from published literature (Prates and Drumond 2007, Trajano and Bichuette 2010, Ázara and Ferreira 2013, 2014, Pel-



legrini and Ferreira 2014, Souza-Silva et al. 2015, ISLA 2015) and after conducting five visits (four people/six hours inside the caves in each visit) to AS and eight visits (four people/six hours inside the caves in each visit) to TG to sample the cave fauna in microhabitats and on organic resources. Extensive visual searching and manual collections were made with the aid of soft forceps and brushes. All microhabitats such as root mats, plant debris, guano deposits, spaces under stones, and humid spots were inspected. In the water (both flowing and still) the invertebrates were collected with the aid of tweezers and hand nets (Ferreira 2004, Souza Silva et al. 2011, Simões et al. 2015). The collection team was always composed of four biologists with at least three years of experience in caving and manual collection of invertebrates. Zooplankton nets were not used for sampling fauna from any cave habitat. The epikarst/vadose zone species were collected from travertine pools formed by dripping waters, located in areas not subject to the river flooding (as the case of *Hyaella epikarstica* Rodrigues, Bueno & Ferreira, 2014).

The determination of potentially new troglobitic or stygobitic species was made by identifying “troglomorphisms” in the unknown sampled specimens (when evident), by consulting the specialist researcher of the group or considering those already determined and described in the literature (Tables 1 and 2, Figures 2 and 3).

Pitfall traps were not used because of their low efficiency that has already been demonstrated in some studies conducted in Neotropical caves (Weinstein and Slaney 1995, Sharratt et al. 2000). Moreover, such traps can cause disturbances in cave populations (Ferreira 2004, Souza-Silva et al. 2011).

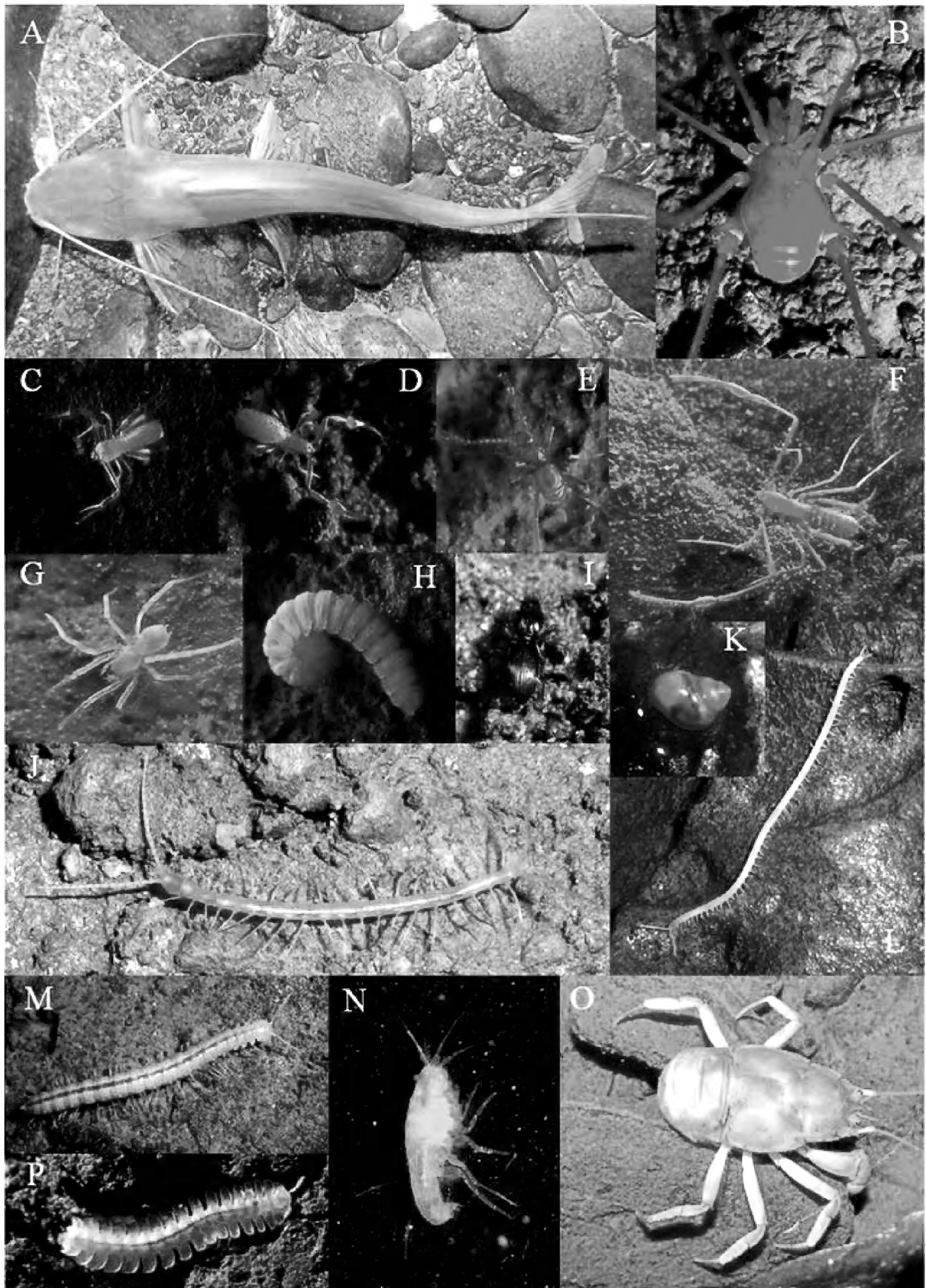
All collected organisms were fixed in 70% ethanol, identified to an accessible taxonomic level, grouped into morphospecies (Souza-Silva et al. 2011), and deposited in the Subterranean Invertebrate Collection (ISLA) of the Center of Studies on Subterranean Biology in the Biology Department at the Federal University of Lavras (CEBS-UFLA).

### **Organic debris availability**

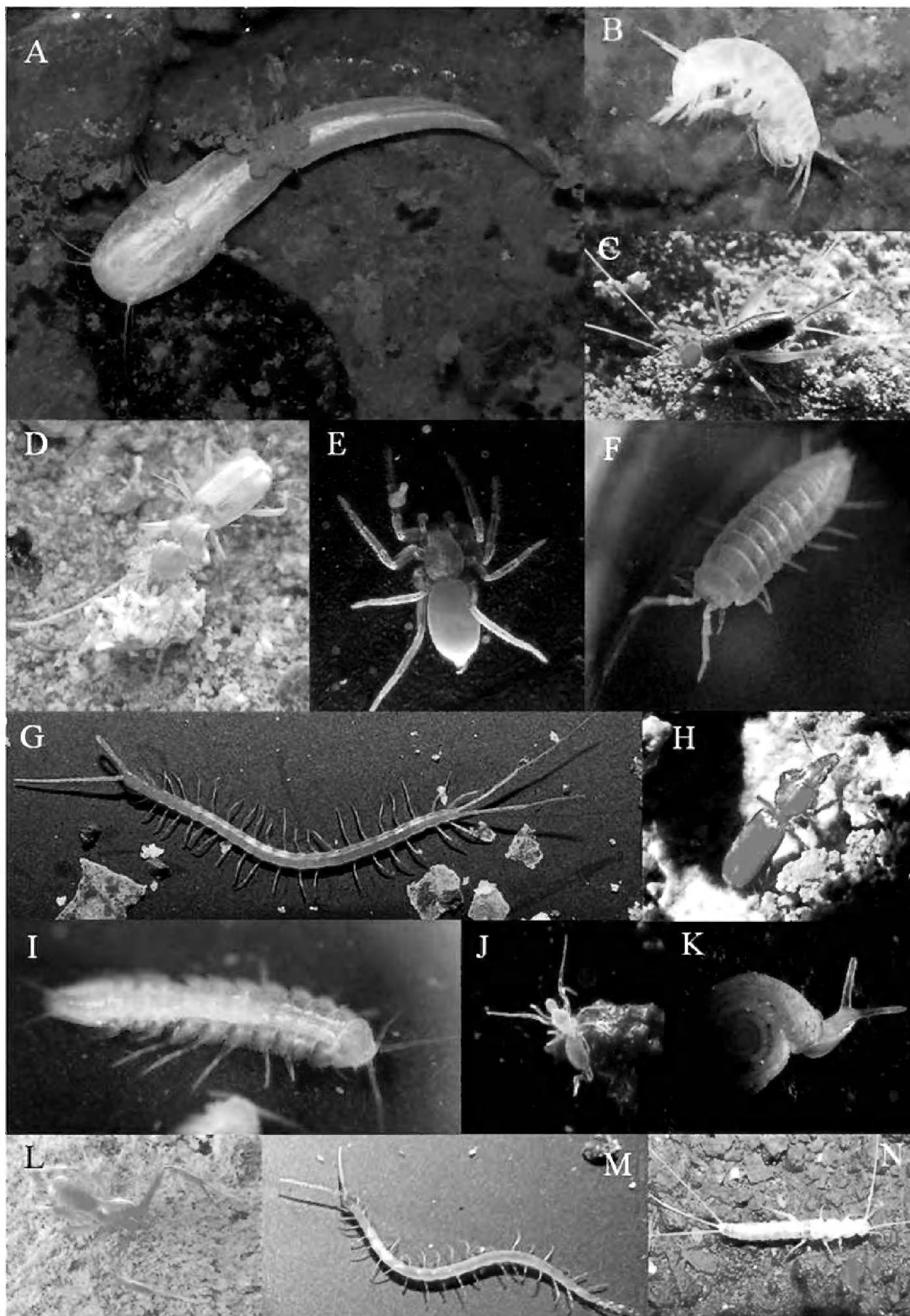
The organic resources visible in the caves were examined (*in situ*) to describe the possible food resources for the fauna. The information regarding quantification of resources, their access pathways, accumulation, and decomposition were not gathered. The trophic characterization was thus restricted to the qualitative aspects.

### **Human impacts**

During the visits to the caves and using satellite images available on Google Earth 2014, we rated human impacts present inside and around the caves to a maximum distance of 250 m in accordance with the Brazilian legislation (Souza-Silva et al. 2015). Besides, one of the authors (R.L. Ferreira) has often visited the Toca do Gonçalves and its surroundings since 1996.



**Figure 2.** Some of the stygobiotic and troglobitic species in Areias cave system, São Paulo, Brazil. *Pimelodella kronei* (A), *Pachylospeleus strinatii* (B), *Pseudochthonius strinatii* (C), *Ideoroncus cavicola* (D), *Pselaphidae* sp. n. (E), *Spelaeobochica muchmorei* (F), *Hahniidae* sp. n. (G) *Cryptodesmus* spn (H), *Schizogenius ocelatus* (I), *Cryptops iporangensis* (J), *Potamolithus troglobius* (K), *Crypturodesmus* spn (L), *Leodesmus yporangae* (M), *Hyallela epikarstica* (N), *Aegla cavernicola* (O), *Peridontodesmella* sp. (P).



**Figure 3.** Some of the stygobiotic and troglobitic species in Toca do Gonçalves. *Rhandiopsis* sp. n. (A), *Spelaeogamarus trajanoae* (B), Phalangopsidae sp. n. (C), *Coarazuphium caatinga* (D), *Lygromma* sp. n. (E), Scleropactidae sp. n. (F), *Newportia spelaea* (G), Clivinina sp. n. (H), *Pongycarcinia xyphidiorus* (I), *Allokoenenia* sp. n. (J), *Rotadiscus* sp. n. (K), Cthoniidae sp. n. (L), Geophilomorpha sp. n. (M), Nicoletiidae sp. n. (N).



**Table 1.** Composition of invertebrate and vertebrate obligate cave fauna of the Areias cave system in different habitats. Terrestrial habitat (T), aquatic habitat (A), Epikarst/vadose zone (E/V).

Taxon	Taxon	Family	Species/morphospecies	Habitat
Arachnida	Araneae	Hahniidae	Hahniidae spn	T
	Opiliones	Gonyleptidae	<i>Pachylospeleus strinatii</i>	T
	Palpigradi	Eukoeneniidae	<i>Eukoenenia</i> spn	T
	Pseudoscorpiones	Bochicidae	<i>Spelaebochica muchmorei</i>	T
		Chthoniidae	<i>Pseudochthonius strinatii</i>	T
		Ideoroncidae	<i>Ideoroncus cavicola</i>	T
Crustacea	Decapoda	Aegliidae	<i>Aegla cavernicola</i>	A
	Amphipoda	Hyallegidae	<i>Hyallegella epikarstica</i>	E/V
	Isopoda	Phylosciidae	<i>Benthana iporangensis</i>	T
		Platyarthridae	<i>Trichorhina</i> sp. n.1	T
			<i>Trichorhina</i> sp. n.2	T
Hexapoda	Coleoptera	Carabidae	<i>Schizogenius ocelatus</i>	T
		Pselaphidae	Pselaphidae sp. n.	T
	Collembola	Cyphoderidae	Cyphoderidae sp. n.	T
		Isotomidae	cf. <i>Folsomia</i> sp. n.	T
		Paronellidae	<i>Troglolaphysa aelleni</i>	T
			Paronellidae sp. n.	T
	Diplura	Campodeidae	<i>Oncinocampa trajanoae</i>	T
Mollusca	Gastropoda	Hydrobiidae	<i>Potamolithus troglobius</i>	A
Myriapoda	Chilopoda	Cryptopidae	<i>Cryptops iporangensis</i>	T
		Geophilidae	Geophilidae sp. n.	T
	Polydesmida	Chelodesmidae	<i>Leodesmus yporangae</i>	T
		Cryptodesmidae	<i>Cryptodesmus</i> sp. n.	T
			<i>Peridontodesmella</i> sp.	T
		Oniscodesmidae	<i>Crypturodesmus</i> sp. n.	T
Nemertea	Nemertea	-	Nemertea sp. n.	A
Osteichthyes	Siluriformes	Heptapteridae	<i>Pimelodella kronei</i>	A
Platyhelminthes	Turbellaria	Dugesidae	Dugesidae sp. n.	E/V

Data analysis

The taxonomic diversity ( $\Delta$ ) was calculated for both the AS and TG caves. This index reflects the average taxonomic distance between taxa (Pienkowski et al. 1998). The richness was divided by the cave length in Areias de Cima, Areias de Baixo, Ressurgência das Areias, and Toca do Gonçalo in order to obtain a standardized richness (Table 3). Finally, an accumulation curve was performed for each cave. However, since the sampling methods and efforts used to access the fauna were historically quite distinct, the curves are actually graphic representations of the descriptions dates (data from literature) and the records of species sampled in each cave (new records here presented, Figure 4).



**Table 2.** Composition of invertebrate and vertebrate obligate cave fauna of the Toca do Gonçalves cave, in different habitats. Terrestrial habitat (T), aquatic habitat (A).

Taxon	Taxon	Family	Species/morphospecies	Habitat
Arachnida	Araneae	Prodidomidae	<i>Lygromma</i> sp. n.	T
	Palpigradi	Eukoeneriidae	<i>Allokoeneria</i> sp. n.	T
	Pseudoscorpiones	Cthoniidae	Cthoniidae sp. n.	T
Crustacea	Amphipoda	Artesiidae	<i>Spelaeogammarus trajanoae</i>	A
	Isopoda	Calabozoidae	<i>Pongycarcinia xyphidiorus</i>	A
		Styloniscidae	Styloniscidae sp. n.	A
		Scleropactidae	Scleropactidae sp. n.	T
		Platyarthridae	<i>Trichorhina</i> sp. n.	T
Hexapoda	Coleoptera	Carabidae	Clivinina sp. n.	T
			<i>Coarazuphium caatinga</i>	T
		Ditycidae	Ditycidae sp. n.	A
	Collembola	Arrhopalitidae	<i>Arrhopalites</i> sp. n.	T
	Ensifera	Phalangopsidae	Phalangopsidae sp. n.	T
	Sternorrhyncha	Ortheziidae	Ortheziidae sp. n.	T
	Zygentoma	Nicoletiidae	Nicoletiidae sp. n.	T
Mollusca	Gastropoda	Charopidae	<i>Rotadiscus</i> sp. n.	T
Myriapoda	Chilopoda	Cryptopidae	<i>Cryptops spelaeoraptor</i>	T
		-	Geophilomorpha sp. n.	T
		Scolopocryptopidae	<i>Newportia spelaea</i>	T
	Polydesmida	Oniscodesmidae	Oniscodesmidae sp. n.	T
	Polyxenida	-	Polyxenida sp. n.	T
Osteichthyes	Siluriformes	Heptapteridae	<i>Rhandiopsis</i> sp. n.	A

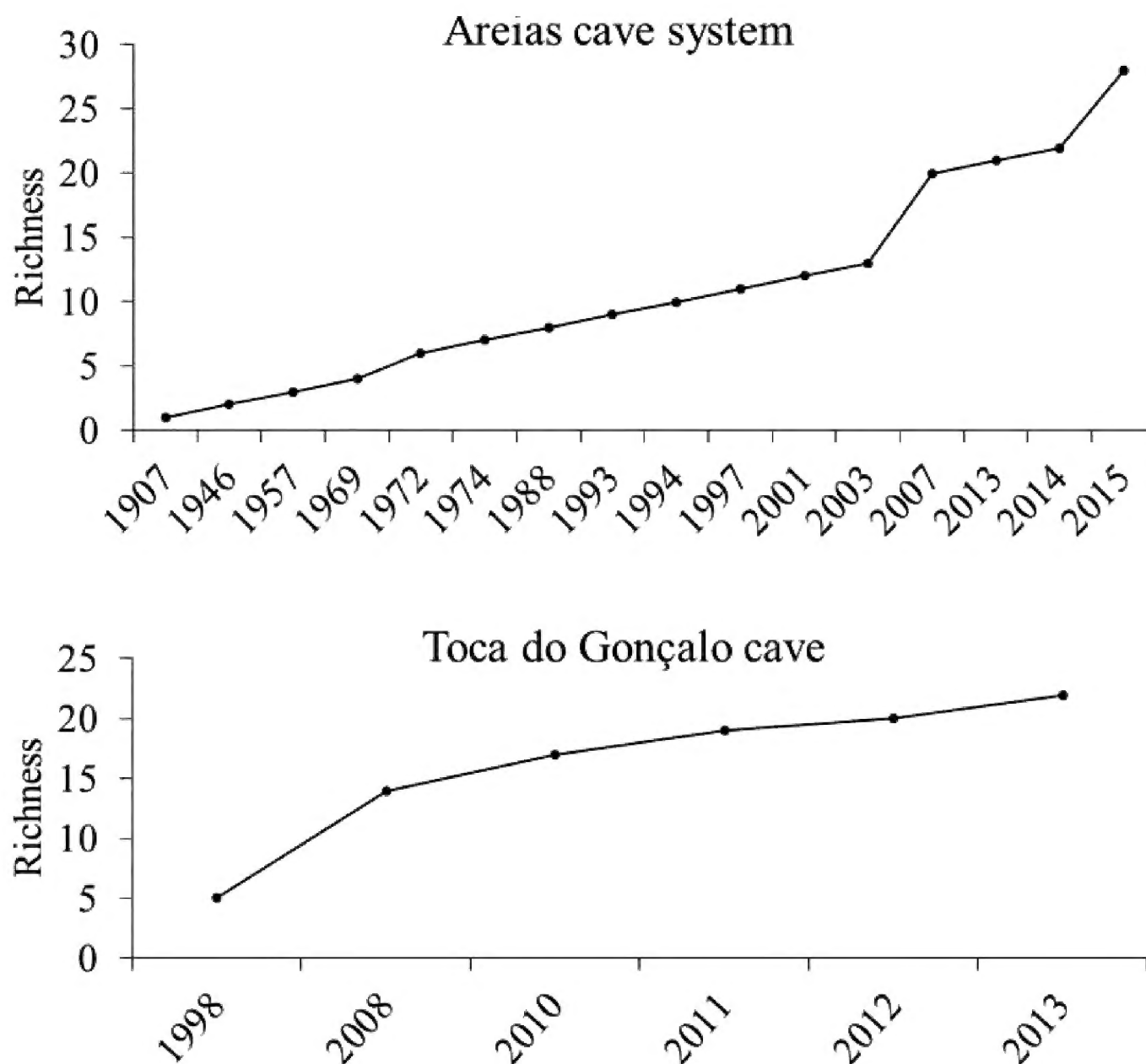
**Table 3.** Proportional number of species in Areias caves and Toca do Gonçalves cave in relation to cave extension.

Cave	Extent (m)	Number of Species	Species/m
Areias de cima	5500	19	0.003
Areias de baixo	1500	11	0.007
Ressurgência das areias	1200	7	0.005
Toca do Gonçalves	500	22	0.044

## Results

### General faunal composition and richness (AS and TG)

A total of 50 troglobitic/stygobitic species was recorded in both systems (28 species in Areias caves system and 22 species in Toca do Gonçalves cave). These taxa belong to the Platyhelminthes (1 sp.), Nemertea (1 sp.), Gastropoda (2 spp.), Amphipoda (3), Isopoda (7), Decapoda (1), Collembola (5), Coleoptera (5), Ensifera (1), Sternorrhyncha (1), Zygentoma (1), Diplopoda (6), Chilopoda (5), Araneae (2), Opiliones (1), Palpigradi (2), Pseudoscorpiones (4), and Osteichthyes (2). Both caves have perennial



**Figure 4.** Species accumulation curves for the two Brazilian hotspots. The time-scale do not present regular intervals. Such curves were performed considering both data from literature and the new records here presented.

water bodies, but terrestrial invertebrates are dominant in numbers of taxa for both systems (77%). At least 32 invertebrate families and one vertebrate family (Pisces: Heptapteridae) representing the obligate cave fauna are present in both caves (Tables 1 and 2). Although the two caves have 50 troglobitic species together, only 19 species are described. The taxonomic distinctness of the Areias system was 4.9, and 4.8 in Toca do Gonçalves, with no significant difference between the two systems. The species number accumulation curves for each cave are presented in Figure 4.

### **Areias cave system**

#### **Faunal composition, richness and distribution**

The Areias cave system has at least 28 obligate cave species belonging to eight higher taxa and 25 families. The eight higher taxa were Hexapoda (7 spp.), Myriapoda (6),

Arachnida (6), Crustacea (5), Mollusca (1), Nemertea (1), Osteichthyes (1), and Platyhelminthes (1) (Table 1). The richness of the observed obligate cave fauna was Turbellaria (1 sp.), Nemertea (1), Gastropoda (1), Isopoda (3), Amphipoda (1), Decapoda (1), Polydesmida (4), Chilopoda (1), Geophilomorpha (1), Collembola (4 spp.), Coleoptera (2), Diplura (1), Pseudoscorpiones (3), Araneae (1), Opiliones (1), Palpigradi (1), and Siluriformes (1). Only 50% of the obligate cave species from the Areias cave system are previously described as most of them live in terrestrial habitats (77%). They are *Potamolithus troglobius* Simone & Moracchioli, 1994, *Aegla cavernicola* Turkey, 1972, *Hyallela epikarstica* Rodrigues, Bueno & Ferreira, 2014, *Benthana iporangensis* Lima & Serejo, 1993, *Cryptops iporangensis* Ázara & Ferreira, 2013, *Leodesmus yporangae* (Schubart, 1946), *Pachylospeleus strinatii* Silhavy, 1974, *Spelaeobochica muchmorei* Andrade & Mahnert, 2003, *Pseudochthonius strinatii* Beier, 1969, *Ideoroncus cavicola* Mahnert 2001, *Schizogenius ocelatus* Whitehead, 1972, *Troglolaphysa aelleni* Yoshii, 1988, *Oncinocampa trajanoae* Conde, 1997, and *Pimelodella kronei* (Ribeiro, 1907). The aquatic lotic habitat (4 spp) and epikarst/vadose zone (2 spp.) harbor the remaining species.

### Organic debris

The major sources of nutrients for terrestrial and aquatic fauna in lotic habitat seem to be plant debris. However, there are some biofilms covering the rocks in the river that may be used as food by some benthic invertebrates. Dissolved and particulate organic matter is probably derived from the surface forest. Few carcasses were observed during our surveys in the system (frogs, rats, etc.). In all cases, those carcasses were full of invertebrates such as flies (Phoridae), millipedes (*Pseudonannolene strinatii* Mauriès, 1974), and springtails. Finally, some guano deposits also provide nutrition, especially to terrestrial scavenging invertebrates.

### Human impacts

Currently the Areias system is partially contained within a state conservation unit (Parque Estadual Turístico do Alto Ribeira - PETAR); however, the caves of the system have been submitted to numerous impacts and currently, conflicts related to tourism, land ownership and use in the region still occur. Sporadic visits to the caves are still conducted in an uncontrolled manner in the Ressurgência das Areias cave. The administrator of the reserve (PETAR) coordinates the activities carried out in the upper and lower Areias cave system. The visitation is allowed only for small groups of researchers with previously registered and authorized activities.

Near the Areias caves system trails, deforested areas, houses, domestic animals, and garbage, among other impacts are visible. All these alterations may affect directly or indirectly the groundwater compartments and the fauna. Another fact to consider is that although Areias system is located inside the PETAR, the springs of all the system drainages are located outside the Park and therefore susceptible to human impacts.

## Toca do Gonçalves Cave

### Faunal composition, richness, and distribution

The TG cave fauna comprises at least six higher taxa, 22 obligate cave species, and 18 families. The six higher taxa were Mollusca (1 sp.), Crustacea (5 spp.), Myriapoda (5), Hexapoda (7), Arachnida (3), and Osteichthyes (1) (Table 2).

The richness of the observed obligate cave fauna were Gastropoda (1 sp.), Isopoda (3 spp.), Amphipoda (2), Polyxenida (1), Chilopoda (3), Palpigradi (1), Polydesmida (1), Araneae (1), Pseudoscorpiones (1), Coleoptera (3), Collembola (1), Ensifera (1), Zygentoma (1), Sternorrhyncha (1), and Siluriformes (1). Contrary to what was observed for the Areias caves system, only 22% of the obligate species from TG cave are only recently described and most of them live in terrestrial habitats (77%). These are *Spelaeogammarus trajanoae* Koenemann & Holsinger, 2000, *Pongycarcinia xyphidiorus* Messana, Baratti & Benvenuti, 2002, *Cryptops spelaeoraptor* Ázara & Ferreira, 2014, *Newportia spelaea* Ázara & Ferreira, 2014, and *Coarazuphium caatinga* Pellegrini & Ferreira, 2014.

### Organic debris

The major sources of nutrients for terrestrial and aquatic fauna seem to be dissolved and particulate organic matter that comes from the epigean habitats; however, there are some root mats in the water table level that could be used as food. During the rare rainy seasons, the water runoff from epigean environments can transport coarse and fine particulate organic matter underground. Small guano deposits also occur in the cave and are mainly used by springtails.

### Human impacts

The TG cave is facing numerous human impacts, the main one resulting from water extraction. For decades, the villagers of Gonçalves drew from the cave water for their subsistence. This removal was initially done manually but subsequently a diesel pump was placed inside the cave, thus reducing the level of the water table significantly, drying out previously flooded areas of the cave. This pump releases a lot of waste, such as the oil that directly contaminated the soil and the water. Such residues were released for at least 20 years (Prevorčnik et al. 2012, R. L. Ferreira pers. comm.). In 2010 an electric pump was installed in the cave. According to the villagers, this pump was used to remove water from the cave daily. In one of our surveys this pump was working during all the time we were inside the cave. According to the villagers, this water was used by a local farmer for irrigation (tomato and green pepper). The worrisome fact is the pronounced reduction observed in the base level (at least two meters). In 2012 there was neither withdrawal of water or equipments inside the cave because the CECAV (a Federal government agency that deals exclusively with cave matters), intervened and petitioned the municipal government to dig an artesian well for the village of Gonçalves. However, although the water is no longer been extracted from the cave, it is been



extracted from the water table, thus reducing the water level in the whole water table, what is reducing the water level in the cave areas previously flooded. During the visit to the cave in August 2013 a major reduction in the water level was observed (approximately 3 m compared to the “normal” level), but this might be due also to the long drought period in the region (three years without heavy rainfall).

## **Discussion**

The Brazilian cave fauna began to be relatively well studied from 1980 onwards (Dessen et al. 1980); however few caves have been intensively studied. Systematic inventories in the presented caves (AS and TG) have revealed a high diversity of troglobitic species and good potential to discover new species. However, in the studies performed to date on the fauna from more than 2,000 Brazilian caves with approximately 800 troglomorphic/troglobitic species, only the Areias and Toca do Gonçalo systems occupy a prominent position in relation to the number of stygobiotic and troglobitic species (Trajano and Bichuette 2010, Souza-Silva et al. 2015, Cordeiro et al. 2014, Gallão and Bichuette 2015). In addition, TG stands out with 22 troglobitic species found in both terrestrial and aquatic habitats because of its reduced extension and depth (Table 3). Until present, only one cave with small extensions and many recorded troglomorphic species is known in Brazil (MP8, an iron ore cave that has 14 obligate cave species) (Souza-Silva et al. 2011). MP8 cave is a 128 m long cave, although it may be longer if we consider the probable sub-surface system of small space (canaliculi) that make up an extensive network of interstitial spaces (meso- and micro-caves) connected to the macro-caves, making for ferruginous subterranean system habitats with great extensions (Ferreira 2005, Souza-Silva et al. 2011, Hoch and Ferreira 2012).

## **Accessing the subterranean biodiversity**

Toca do Gonçalo cave was less studied than other cave systems in Brazil. The Areias system has been studied for more than 100 years, and its most recently discovered troglobitic species were collected and described by Rodrigues et al. in 2014. The importance of systematic studies for an exhaustive faunal characterization of subterranean system has other clear examples in the world (Deharveng and Bedos 2012, White and Culver 2012). Thus, besides the relevance of spatial accuracy of the search and methods used to collect the fauna, the frequency of sample collections can directly influence the observed richness (Gallão and Bichuette 2015). It is difficult to assume that a macro- or meso-subterranean habitat had its species richness exhausted: new searches will almost always be necessary. As indicated in the species accumulation curves, in both systems the curves did not reached an asymptote, indicating that there are more species to be discovered in the future. The difficulty in sampling subterranean environments are related to the inaccessibility of fissures, mesocaverns, and interstitial habitats

(Culver and Pipan 2009, Trontelj et al. 2012, Ortunó et al. 2013). Thus, there is a clear need for successive collections when one intends to effectively document the subterranean biodiversity. However, we must consider that for comparative studies, methods of rapid evaluation may be used successfully (Souza-Silva et al. 2015, Simões et al. 2015), provided a standardized sampling method has been used. In this case, we can expect that caves with many troglobitic species stand out in relation to the others, even with few collection events. As an example, during our first collection in TG, 63% of the total species currently known was obtained, and such a cave stood out from all the other caves that had been inventoried in the same area. The lack of subterranean biodiversity hotspots in South America may be at least partially attributed to the lack of systematic studies in this continent. Furthermore, the lack of standardized sampling methods may also have hampered the establishment of areas requiring priority for conservation. Additionally, the hotspot concept is somewhat restrictive since it considers, at most, interconnected caves and/or cave systems. In Brazil, although some karstic regions (Pains, Carajás, Apodi, etc.) present high richness of obligate cave fauna, any cave could harbor alone more than 20 species (CEBS-UFLA 2015), as suggested by Culver and Sket (2000). Then, in order to attract attention for sites with representative subterranean communities, we should consider not only caves but the whole representative drainage basin, especially with respect to conservation priorities.

Furthermore, when Culver and Sket (2000) classified the hotspots of subterranean biodiversity they did not consider the degree of threat to which these habitats were submitted, according to the hotspot model proposed by Myers et al. (2000). Places prone to economic activities undergo rapid landscape transformations, and in many cases, these are irreversible. Well-conserved landscapes can quickly be reconfigured as pasture areas or to be destroyed by mining activities. In this sense, the strict use of troglobitic species richness might not necessarily indicate the “health” of a given subterranean system, as this will in fact depend on the type of impact that it has received (Souza-Silva et al. 2015). Accordingly, the degree of impacts to which a cave is threatened should also be incorporated in this concept (as proposed by Myers et al. 2000) and reinforced here, especially considering that conservation policies usually act towards investing in priorities. Richer systems are certainly more threatened, thus deserving priority actions for their conservation (Souza-Silva et al. 2015). In the case of the two hotspots of subterranean biodiversity presented in this work, Toca do Gonçalo certainly should have priority in conservation policies, considering the degree of threat to which it is exposed (even though possessing fewer troglobitic species than the Areias cave system, which is already, at least partially, protected).

### **Global relevance of the two Neotropical hotspots of subterranean biodiversity**

Compared to other places in the world, South America stands out with only 5% of the 38 subterranean biodiversity hotspots, similar to southeastern Asia and Australia. The regions with higher densities and more species of aquatic and terrestrial obligate cave fauna are in

Europe (47% of the hotspots), North America, and the Canary Islands (15.7% each). Besides geographic influences on the distribution of subterranean hotspots, the cave extension (caves with more than 5 km of extension) and high productivity (chemoautotrophic production, roots, debris) are also important for determining the biodiversity.

Other explanations for high biodiversity in subterranean habitats are cases of colonization through adaptive shift in dry caves or permanent groundwater, springs, rivers, shallow subterranean habitats or epikarst (Culver and Sket 2000, Culver and Pipan 2009 and 2013). This is not the cause of diversity, but one path of colonization that could also explain the diversity in other subterranean habitats. Deharveng and Bedos (2012) suggested as one emerging biodiversity pattern in the tropics, that the richness of troglobionts or guanobionts is rarely very low and never null as in temperate regions, affected by glaciations. However, in fact, most of the Brazilian caves do not harbor any troglobitic/guanobitic species. According to those authors, the scarcity in troglobitic species is also observed in other countries of tropical America, except for some areas in Guatemala, Belize, and Mexico, for which dozens of troglobitic species are in fact known (Reddell 1981, Reddell and George 1996). However, it is important to highlight the huge contribution of anchialine and aquatic relict fauna to the richness of subterranean fauna in such countries (Iliffe 1993, Alvarez et al. 2015).

The high number of obligate cave species recently described in Brazil, and hundreds of troglobitic species still waiting to be described, also contradict Deharveng and Bedos (2012), who suggested that the terrestrial fauna appears to be more diverse in the Oriental and Australian regions than in the Neotropics or Africa. The fact that many subterranean species have been discovered in the last years in Brazil does not mean that many Brazilian caves harbor obligate cave species. In fact, from the approximately 2,000 sampled caves in Brazil, only a few hundred are inhabited by obligate cave fauna. The recent discoveries of troglobitic species in Brazil are certainly due to an improvement on the sampling efforts in many previously non-prospected areas in Brazil (Prevorčnik et al. 2012, Ázara and Ferreira 2013 and 2014, Fišer et al. 2013, Iniesta et al. 2012, Iniesta and Ferreira 2013, 2013a, 2013b and 2015, Bastos-Pereira and Ferreira 2015, Vasconcelos and Ferreira 2016).

Another situation that was mentioned by Deharveng and Bedos (2012) is the absence of MSS (“Milieu Souterrain Superficiel”, Juberthie and Decu 1994) in the tropics. However, in Brazil, the canaliculi systems of ferruginous rocks may well function as an MSS, also influencing the isolation and diversification of the subterranean fauna in the tropics (Ferreira et al. 2015), but these shallow habitats are restricted to ferruginous rocks (Souza-Silva et al. 2011) which represent 0.15 % of the Brazilian territory, nearly 12,000 km<sup>2</sup> (Vieira et al. 2015).

### **Conservation and protection of the two Neotropical hotspots**

The AS and TG undergo human alterations from past and present activities and deserve special and urgent attention regarding further research and protection.

In 1970 the speleologist Guy Collet installed water tanks within the Ressurgência das Areias Cave in order to construct the first subterranean laboratory in Brazil (Trajano 2007). In addition, G. Collet installed a plastic curtain at the cave entrance to prevent the external light to access the inner portions of the cave, even near the entrance. However, the curtain installation affected the transit of bats in the cave and therefore affected the guano production. To populate the tanks, he collected dozens of blind catfish (*Pimelodella kronei* Ribeiro, 1907) that did not survive. The subterranean laboratory was disabled some years later.

Surrounding the Parque Estadual Turístico do Alto Ribeira (PETAR) there are land occupation and poorly planned land uses, in addition to non-controlled visitation endangering not only the surface land systems, but also the integrity of the associated hydrologic systems, including the subterranean environment (Genthner et al. 2007). Protecting the subterranean fauna also requires the protection of the surface riparian areas, including the upstream cave sources (Ford 2005, Reboleira et al. 2011, Pellegrini et al. 2016). Protection of deep-groundwater species requires protection from both excessive draw further down the system and contamination of the aquifer (Culver and Pipan 2013). Protecting the troglobites that rely on the flow of organic matter via the cave entrance includes the protection of the entrance area and any foraging area of the species responsible for bringing organic matter into the cave (Souza-Silva et al. 2011a, Culver and Pipan 2013, Prous et al. 2015).

Terrestrial habitats of the biodiversity hotspots generally contain large numbers of species with small ranges that are potentially vulnerable to global extinction. In these systems, site-specific conservation efforts are a justifiable priority (Hughes et al. 2002, Reboleira et al. 2011, Souza-Silva et al. 2015).

## Final considerations

The tropical region, with both its diversity of rocks and subterranean habitats, has a high potential for finding new biodiversity hotspots. Although only two hotspots have been identified in Brazil until now in this paper, many others may exist and perhaps they are even more threatened or near-threatened in the near future. Besides the lack of researchers and investment in cave studies in Brazil, almost half of the studied subterranean environments were sampled only once and the collection methods were restrict to the terrestrial fauna. Furthermore, there are many karst regions in Brazil with a high richness of obligate cave fauna which desperately deserve attention.

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